### **PART I - ADMINISTRATIVE**

Section 1. General administrative information				
Title of project				
Develop New Feeds Fe	or Fish Used In Recovery And Restoration Efforts			
BPA project number: Contract renewal date (n	20105 nm/yyyy): Multiple actions?			
Business name of agency Abernathy Salmon Culture	, institution or organization requesting funding Technology Center			
Business acronym (if app	propriate) <u>USFWS</u>			
Proposal contact person	or principal investigator:			
Name	Ann Gannam			
Mailing Address	1440 Abernathy Creek Rd.			
City, ST Zip	Longview, WA 98632			
Phone	360-425-6072			
Fax	360-636-1855			
<b>Email address</b>	Ann_Gannam@mail.fws.gov			
NPPC Program Measure Number(s) which this project addresses 7.2, 7.2 A.6, 7.2D, 7.2D3, 7.2D4, 7.4A.1, 7.4D				
FWS/NMFS Biological C	Opinion Number(s) which this project addresses			
Other planning documer	nt references			
Short description Formulate new diets to impose	prove the health and condition of propagated salmonids			
Target species Spring chinook, coho, stee	elhead			

# Section 2. Sorting and evaluation

## Systemwide **Evaluation Process Sort CBFWA** caucus **Special evaluation process ISRP** project type Mark one or more If your project fits either of Mark one or more categories caucus these processes, mark one or both X Anadromous Multi-year (milestone-Watershed councils/model based evaluation) fish watersheds Resident fish Watershed project Information dissemination Wildlife evaluation Operation & maintenance New construction Research & monitoring Implementation & management Wildlife habitat acquisitions Section 3. Relationships to other Bonneville projects Umbrella / sub-proposal relationships. List umbrella project first. Project # Project title/description Other dependent or critically-related projects Project # **Project title/description Nature of relationship** Section 4. Objectives, tasks and schedules Past accomplishments Met biological objectives? Year Accomplishment

### Objectives and tasks

Obj		Task	
1,2,3	Objective	a,b,c	Task
1	Determine mineral requirements	a	Feeding trial
1		b	Disease and salt water challenge
1		c	Tissue analysis
2	Determine appropriate levels of	a	Feeding trial
	lipid and carbohydrate for diets		
2		b	Disease and salt water challenge
2		c	Tissue analysis
3	Formulate, produce and test new	a	Make feed
	diet		
3		b	Feeding trial
3		c	Disease and salt water challenge
3		d	Tissue analysis
3		e	Check for quality after storage

### Objective schedules and costs

Obj#	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	10/1999	11/2000	selected mineral		50.00%
			requirements determined		
2	10/1999	11/2000	energy level determined		50.00%
3	11/2000	9/2001	new diet developed		
				Total	100.00%

### **Schedule constraints**

Availability of fish needed for the studies may cause a time constraint

### **Completion date**

9/2001

# Section 5. Budget

### FY99 project budget (BPA obligated):

### FY2000 budget by line item

Item	Note	% of total	FY2000
Personnel	WG-7 Extruder Operator/Fish Culturist	%31	31,180
Fringe benefits		%9	9,354
Supplies, materials, non- expendable property	Feed ingredients, chemicals, lab supplies for fish and feed analyses	%32	32,500
Operations & maintenance	Operating well, maintenance of equipment	%4	4,000
Capital acquisitions or			
improvements (e.g. land,			
buildings, major equip.)			
NEPA costs			
Construction-related			
support			
PIT tags	# of tags:		
Travel			
Indirect costs		%22	22,427
Subcontractor			
Other	uniform allowance	%0	300
TOTAL BPA FY2000 BUDGET REQUEST			\$99,761

### Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount (\$)
	Total project cost (inclu	ding BPA portion)	\$99,761

### Outyear costs

	FY2001	FY02	FY03	FY04
Total budget	\$67,100			

### Section 6. References

Watershed?	Reference
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	Barrows, R. 1997. The effect of diet on fin erosion in rainbow trout.  Presented at the Fish feed and Nutrition Workshop, Frankfort, KY, September
	21-23, 1997.
	Bell, J. G. and C. B. Cowey. 1989. Digestibility and bioavailability of dietary selenium from fishmeal, selenite, selenomethionine and selenocystine in Atlantic salmon (Salmo salar). Aquaculture 81:61-68.
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	Caceres-Martin, C. M. Cadena-Roa and R. Metailler. 1984. Nutritional requirements of turbot (Scophthalums maximus): I. A preliminary study of protein and lipid utilization. Journal of the World Mariculture Society 15:191-202.
	Castell, J. D., J. G. Bell, D. R. Tocher and J. R. Sargent. 1994. Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (Scophthalmus maximus
	Clarke, W. C. and J. Blackburn. 1978. Seawater challenge tests performed on hatchery stocks of chinook and coho salmon in 1977. Fisheries and Marine Service Technical Report 761. Pacafic Biological Station, Nanaimo, British Columbia V9R 5K6.
	Dupree H. F. Cauglitz Ir. A. Hall and C. Haula. 1070. Effects of distance
	Dupree, H., E. Gauglitz, Jr., A. Hall and C. Houle. 1979. Effects of dietary

lipids on the growth and acceptability (flavor) of channel catfish (Ictalurus punctatus). Proc. World Symp. on Finfish Nutrition and Fishfeed Technology, Hamburg 2:87-103.
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1
Felton, S. P., R. Grace and M. Landolt. 1994. Significantly higher levels of zinc and copper found in wild compared to hatchery-reared coho salmon smolts Oncorhynchus kisutch. Diseases of Aquatic Organisms. 18:233-236.
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Gannam, A. L. 1997. Development of open formula diets and new feeding stategies: A progress report. 48 <sup>th</sup> Annual Pacific Northwest Fish Culture Conference, Gleneden Beach, OR, December 2-4, 1997.
Heck, N. E. and H. E. Calbert. 1977. Use of animal fat in formulated diets for yellow perch. Proceedings of the eighth annual meeting world Mariculture Society; 1977 January 9-13; San Jose, Costa Rica. c1977:787-791.
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Wilson, R. P. 1994. Utilization of dietary carbohydrate by fish. Aquaculture 124:67-80.

# **PART II - NARRATIVE**

# Section 7. Abstract

As recognized in the 1994 Columbia Basin Fish and Wildlife Program, Section 7.2, habitat destruction has brought about the realization that there will be a greater dependence on hatchery raised stocks to replenish fish runs in the Columbia Basin and elsewhere. Nutrition research is essential when hatchery fish will be used for recovery and restoration of weak wild and naturally spawning fish populations. Current hatchery production fish will also benefit from new diets. The semimoist diets contain 17-25% moisture as compared to dry diets (<17% moisture), and moist diets (>25% moisture). The open formula semimoist diet proposed for development will provide a feed that is more palatable to fish than existing dry feeds. Also, semimoist diets are easier and more economical to store than moist feeds. The importance of open formula diets in these rearing programs is twofold. In open formula diets the ingredients are known and can be specified. Also, because the formulas are open, they can be monitored through quality control programs. Thus, new open formula diets need to be developed for use in hatcheries.

Feeding trials conducted in specific areas of nutrition research will include diet energy and mineral requirements. The diet trials will be conducted with three stocks of fish: Carson spring chinook, Big Creek coho, Big Creek steelhead. The fish become available at different times so the studies will be staggered throughout the year. Growth, survival and feed conversion will be monitored during the trials. At the end of the studies, the needed tissue samples will be collected and analyzed. Disease and salt water challenges will be conducted to assess disease response and fish condition.

### Section 8. Project description

#### a. Technical and/or scientific background

The main goal of the project is to develop a new semimoist feed. In order to produce the new feed certain aspects of salmonid nutrition need to be investigated. Development of life stage diets for fry and juvenile fish raised in hatcheries is very important to managers and culturists. Production of new, open formula feeds can help maximize post release survival of hatchery fish. An additional objective would be to maximize the health of the fish released from hatcheries.

As more weak and endangered stocks are moved into captive environments, having feeds developed to meet their requirements will be important. Studies to develop new open formula diets should be initiated. Goals are to produce feeds that yield healthy smolts and that maximize post release survival of juveniles to adulthood.

Levels of fat as well as composition of the fat are important. Dupree et al. (1979) reported that as the lipid content of the diet for channel catfish increased from 0% to 20%, the whole fish lipid levels increased from 3.8% to 13.2%. In a study using turbot, Caceres-Martinez et al. (1984) saw a negative effect in the fish tissue of excess dietary lipids. At the lowest protein level, 37.5%, the tissue lipid deposition increased as the lipid level in the feed increased from 10% to 20%.

Several investigators have shown that the fatty acid content of the lipids used in fish diets are important for good growth (Farkas, et al. 1977, Stickney and Andrews 1972, Heck and Calbert 1977, Farkas, et al. 1977, Stickney et al. 1984). The importance of fatty acids in the correct proportions in the diets is shown in the study done by Lewis et al. (1985). Catfish fed various combinations of stearic, oleic, linoleic, and linolenic acids did poorly. Castell et al. (1994) also showed the importance certain fatty acids in fish feeds. They found that arachidonic acid (20:4n-6) may be an essential fatty acid for juvenile turbot. Arachidonic acid is not give much attention in fish diets, however, Bell et al. (1994) found in the 10 freshwater invertebrates (common prey for salmon) he analyzed for fatty acid composition that there were higher levels of 18:2n-6, 18:3n-3, 20:4n-6 and 20:5n-3 and less 22:6n-3 than found in commercial diets used in smolt production. In a study done Lie et al. (1986), three different fats were tested in diets for cod. They used cod liver oil, Greenland halibut oil and peanut oil. The study suggested that the type and amount of fat used influenced fat deposition in the liver.

The liver is an indicator of how good the diet is for the fish. Fowler and Wood (1966) tested different supplemental dietary fats on chinook salmon fingerlings. The investigators found the when chinook salmon were fed a meat diet or an all-meat diet supplemented with hard animal fat as the principal lipid source, fatty liver and degenerative changes in the spleen and hematopoietic part of the kidney occurred. Use of a vegetable oil instead of animal fat prevented these abnormalities.

In a preliminary study, manipulation of the fat levels in the feed has resulted in significantly lower (P<0.05) total body fat in the fish (Gannam 1997).

Carbohydrates, another energy source, in the diet of salmonids are an important consideration, especially now that extrusion of feeds makes the carbohydrate more digestible. Feeding high levels of digestible carbohydrate to salmon has resulted in increased liver size and glycogen content that is proportional to the carbohydrate fed (Wilson 1994). Starch inclusion in the diet of Atlantic salmon higher than 22% had negative effects on growth and feed use (Hemre et al. 1995). Inclusion of starch above 9% resulted in decreased starch digestibility. Ashley (1972) and Roberts and Bullock (1989) discuss the pathologies of excessive carbohydrates in the diet of fish.

Increasing levels of carbohydrate in the diet appeared to be positively correlated to the levels of glycogen in the liver in a preliminary study (Gannam 1997).

Information on mineral requirements in fish is incomplete because fish can take up minerals from the water through their gills and skin (Lall 1989). Other complicating factors are the availability and digestibility of minerals in the diet (Shearer 1992, Li and Robinson 1996). Work comparing the digestibility chelated and inorganic trace minerals is being done (Bell and Cowey 1989, Li and Robinson 1996, Lim et al. 1996, Paripatananont and Lovell 1997). There have been mixed results in these studies probably depending on the composition of the feed and the alkalinity/hardness of the water.

A study done by Felton et al. (1994) suggests that hatchery raised coho have less copper and zinc content than wild smolts collected in the same watershed. Barrows (1997) showed that the use of a highly digestible chelate of copper improves fin condition in rainbow trout. Water at many hatcheries is low in minerals and diets may be marginally deficient in the minerals needed by fish. Immune function can also be affected by mineral deficiencies (Landolt 1989, Lim et al. 1996, El-Mowafi et al. 1997). For the hatchery fish to be more like their wild counterparts, mineral nutrition needs to be addressed.

#### b. Rationale and significance to Regional Programs

The role of hatcheries in the Pacific Northwest and elsewhere is evolving such that they will become more than mitigation facilities. They will be supplementation and restoration hatcheries for threaten and endangered species (FWP 7.2, 7.2D, 7.2D3, 7.4A1). The feeds developed through this project would help reduce some of the perceived negative characteristics of hatchery raised fish (FWP 7.2A.6, 7.2D, 7.2D4). Without addressing all the variables of the hatchery influence it cannot be determined if hatcheries are fit sources of fish for restoration and recovery of threatened and endangered fish populations.

For many years, fish culturists in the Pacific Northwest have used frozen moist diets to feed hard-to-raise species such as spring chinook salmon. Dry feeds have been used successfully in the culture of many of fishes including trout, steelhead, Atlantic salmon and some species of Pacific salmon. Presently, one manufacturer makes moist frozen feed, and the only open-formula dry feed is made on a compaction mill (California pellet mill). Progress has been made in the salmon feed industry, and cooker-extruders are now being employed at the mills. Several closed-formula extruded feeds are available to hatcheries. However, these feeds have been developed for the commercial aquaculture industry where the desired product is a large fish, produced quickly. Historically hatcheries have also wanted big smolts for release, but thoughts on the "ideal smolt" are changing. A desire exists for hatcheries to produce smaller, leaner fish. The fish would be 'wild' in body composition. To meet this goal, new diets need to be developed. Specific areas of nutrition research would include diet energy and mineral requirements.

#### c. Relationships to other projects

Other projects related to this proposal that are ongoing but not BPA funded are:

Using a copper supplemented diet to reduce dorsal stubbing in sea-run cutthroat trout juveniles with Jack Tipping, Washington Department of Fish and Wildlife

Interactions between *Ceratomyxa shasta* and its salmonid and polychaete host with Jerri Bartholomew, Oregon State University, funded through the USDA Animal Health and Disease Program

Immunomodulators as a fish health management tool with Robin Schrock, USGS, Columbia River Research Laboratory

#### **d. Project history** (for ongoing projects)

(Replace this text with your response in paragraph form)

### e. Proposal objectives

- Determine mineral requirements
   Correct mineral levels will enhance fish health and condition.
- 2. Determine appropriate levels of lipid and carbohydrate for diets Correct levels of lipid and carbohydrate improve fish condition.
- 3. Formulate, produce and test new diet New diet will out perform existing feeds

The product from this study would be a diet for supplementation/enhancement hatcheries.

#### f. Methods

All of the studies to meet the objectives will have a similar format. Three stocks of fish, Carson spring chinook, Big Creek coho, and Big Creek steelhead will be used in these studies. Fish will be stocked into 700 liter circular fiberglass tanks, 250 fish per tank. Four tanks will be randomly assigned to each treatment. Well water (12°C) will be used at 19 liters/minute. The fish will be fed by hand four times a day, five days a week. On the weekends the fish will be fed with automatic feeders four times a day. The amounts of feed fed will be calculated by the method of Buterbaugh and Willoughby (1967). The fish will be weighed every two weeks and the amount feed adjusted accordingly.

Disease challenges will be done using *Vibrio anguillarum* or *Flavobacterium psyphrophilum* using standard challenge protocols. The salt water challenges will be done according to Clarke and Blackburn (1978).

#### **Objective 1: Mineral requirements**

The diets used will be the Abernathy Dry diet with graded levels of each of the test minerals. Each mineral will be tested separately. The control diet will be the Abernathy diet with the standard mineral premix. The mineral controls will contain the minerals in the forms commonly found in mineral premixes. There will be 8 treatments and three replicates. The chelated minerals will be tested against the inorganic minerals traditionally used in feeds. The minerals tested will include: copper, zinc, selenium. For example, the copper will be added as CuSO<sub>4</sub> or chelated copper at 3, 6 or 9 mg/kg diet.

The data reported for the feeding trial will include average weight gain, specific growth rate, gross feed conversion as well as whole body and liver mineral levels. Mineral analyses will be done by Covance, Madison, WI. The data will be analyzed using the one-way analysis of variance to determine if there are differences between treatments (P<0.05). Where differences are found, the treatments that were different from each other will be determined by the Student-Newman-Keuls method (P<0.05) (Ostle and Mensing 1975).

### **Objective 2: Lipid level study**

The treatments will consist of the basic Abernathy diet with different levels of lipid used in the formulation. The levels of lipid used will be 9.0%, 11.4%, 19.9% and the control, 15.7%. Proximate analysis (AOAC 1990) of the feed ingredients for the experimental diets will be determined and the diets formulated. A small compaction-type pellet mill (California Pellet Mill, San Francisco, CA), without steam conditioning, will be used to prepare the diets. Feed will be made at the start of the experiment and stored at room temperature.

The data that will be reported for the feeding trials will include average weight gain, gross feed conversion as well as liver glycogen and liver triglyceride levels. Liver analyses will be done by Biotech Research and Consulting, Inc., Corvallis, OR. Proximate analysis will be done on the fish and the feed. The data will be analyzed using the one-way analysis of variance to determine if there are differences between treatments (P<0.05). Where differences are found, the treatments that are different from each other will be determined by the Student-Newman-Keuls method (P<0.05) (Ostle and Mensing 1975).

#### **Objective 3: Semi-moist diet study**

Three semi-moist formulations and the Abernathy Dry diet as a control will be the treatments. The formulations will have varying levels of carbohydrate added. Proximate analysis (AOAC 1990) of the feed ingredients for the experimental diets will be determined and the diets formulated. A Wenger X85 single screw cooker-extruder will be used to make the feeds. The diets will be made at the start of the experiment and stored at room temperature in plastic containers. The quantities of feed fed to the different treatments will also be corrected for moisture in the feed.

Additionally, a feed storage study will be conducted to check the stability and quality of feed after 5 months of storage. The feed will be collected at the beginning, middle and

end of a production run, stored in a freezer and sampled once a month for five months to detect any degradation. Ascorbic acid, thiamin, folic acid and vitamin  $B_{12}$  will be the vitamins checked. The feed will also be analyzed for peroxide and free fatty acid levels as a check for rancidity. After the storage period of 5 months, fresh feed will be made and both batches of feed will be used in a feeding study. Growth and feed conversion will be the data collected to determine if the nutritional quality of the feed has changed enough to affect the fish. Palatability of the feed will be noted.

The data reported for the feeding trials will include average weight gain, specific growth rate, gross feed conversion. The initial diet trial will require analysis for liver glycogen and liver triglyceride levels. Liver analyses will be done by Biotech Research and Consulting, Inc., Corvallis, OR. The data will be analyzed using the one-way analysis of variance to determine if there are differences between treatments (P<0.05). Where differences are found, the treatments that were different from each other will be determined by the Student-Newman-Keuls method (P<0.05) (Ostle and Mensing 1975).

#### g. Facilities and equipment

Abernathy Salmon Culture Technology Center has 12 raceways, 8' X 80', 104 fiberglass circular tanks, 700 liters (1.2 m diameter, 0.6 m deep), 50 (16 tray) incubator stacks. Water sources include: creek water, 6000 gal/min winter, 3000-4000 gal/min summer, temperatures 4°C-16°C, winter-summer; two wells, old well, 250 gal/min, new well, 400-3200 gal/min, variable speed pump, temperature 12°C±1°. In addition, Abernathy is equipped with a feed preparation laboratory and an analytical laboratory for proximate analyses of feeds and fish. The feed preparation laboratory has a California pellet mill capable of making a compacted pellet at a rate of up to 50 lbs/hour. The laboratory also has a Wenger X85 single screw cooker-extruder that can produce floating, sinking or semi-moist feeds at a rate of 150-600 lbs/hour and all the ancillary equipment needed to make fish feed.

#### h. Budget

The wage grade employee is needed to assist in operation of the extruder and to help care for the study fish. The employee requires an uniform allowance which is the last item in the budget.

The amount requested for the supplies, etc. includes cost for feed ingredients, the price of which varies with the market. Analysis of the fish and feed comprise the majority of the cost. Much of the fish tissue and vitamin analysis is done by Covance Labs or Biotech Research and Consulting.

Money is also request for operations and maintenance. This money will go towards the electricity to run the well. In addition, some of the money will be used to maintain the feed making equipment.

The 22% indirect cost is what the Regional Fish and Wildlife Service Office charges for overhead.

### Section 9. Key personnel

Ann L. Gannam Abernathy Salmon Culture Technology Center Longview, WA 98632

#### **Education**

1988 Ph.D. in Fish Nutrition /Aquaculture, Auburn University, Auburn, Alabama.

1980 Masters of Science in Biology, University of Southern Mississippi,

Hattiesburg, Mississippi.

Bachelor of Science in Zoology, University of Georgia, Athens, Georgia.

#### **Related Experience**

- 1992-present Nutritionist, USFWS, Abernathy Salmon Culture Technology Center. Responsibilities include developing new diets, conducting feeding trials and working on feed problems at the federal hatcheries in Region 1. Am also responsible for feed mill inspections (fish feed quality control) to insure compliance with specifications for feeds made for the federal government in Region 1.
- 1989-1992 Assistant Professor, Fisheries, University of Arkansas Pine Bluff, Department of Agriculture. Was an adjunct assistant professor at the University of Arkansas Fayetteville. Responsibilities included teaching fisheries courses as well as conducting research in fish. Conducted fish nutrition studies addressing alternative protein options for channel catfish, lipid concerns in hybrid striped bass, cost effective feed for golden shiners and temperature/growth for tilapia.
- 9/1988-2/1989 Research Associate in fish nutrition at the University of Arkansas Pine Bluff. Responsibilities included equipping and maintaining the nutrition laboratory. Did preliminary studies to determine the feasibility of using sunflower seed meal as a substitute for soybean meal in catfish diets. Consulted with catfish and baitfish farmers about problems concerning fish feeding and nutrition.
- Gannam, A. L., P. R. Waterstrat, R. Pascho and C. McKibben. 1994. An evaluation of feather meal as a feed ingredient and immunomodulator in fall chinook salmon (*Oncorhynchus tshawytscha*). Poster presented at the International Fish Physiology Symposium, Vancouver, BC, July 16-21, 1994.
- Gannam, A. L. and M. Mauel. 1995. Effects of dietary iron on disease resistance in fall chinook salmon. Abstract, World Aquaculture Meeting, San Diego, CA, February 1-4 1995.
- Schrock, R. M. and A. Gannam. 1996. Comparison of three glucan preparations as feed additives in juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) challenged with *Vibrio*

- *anguillarum.* Presented at the American Fisheries Society Fish Health Section Meeting, Madison, WI, August 6-9 1996.
- Gannam, A. L., R. M. Schrock and M. W. Hack. 1997. The use of three glucan preparations as feed additives in diets for fall chinook salmon, *Oncorhyncus tshawyscha*. Poster presentation at the World Aquaculture Meeting, Seattle, WA, February 19-23 1997.
- Gannam, A. L. 1997. Development of open formula diets and new feeding strategies: A progress report. 48th Annual Pacific Northwest Fish Culture Conference, Gleneden Beach, OR, December 2-4, 1997.

### Section 10. Information/technology transfer

Information generated from this project may be disseminated through peer-reviewed technical journals such as <u>Journal of Nutrition</u>, <u>Journal of Food Science</u>, <u>Aquaculture</u>, <u>The North American Journal of Aquaculture</u> and the <u>Journal of the World Aquaculture</u> <u>Society</u>; the Technology Transfer Series and BPA reports. In addition, feed mills will be notified of positive results and recommendations will be made for feed manufacture.

### Congratulations!